## SAS4305 Analytical Chemistry for Chemical Industry (AS114105/1)

# Chapter 4
# Ultraviolet-Visible (UV-Vis) Molecular Absorption Spectrometry

References:


- Skoog, D.A., Crouch, S.R., Holler, F.J., West, D.M. (2014). Fundamentals of Analytical Chemistry, 9th edition, Brooks/Cole, Chapters 24, 25 & 26.
- Skoog, D.A., Holler, F.J., Crouch, S.R. (2018). Principles of Instrumental Analysis, 7th edition, Thomson, Chapters 13 & 14.


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## Table of Content



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## 4.1 Introduction to molecular spectroscopy

In Molecular spectroscopy,

- the sample solution ***absorbs electromagnetic radiation*** from an appropriate source,
  - and the amount **absorbed is related to the concentration** of the analyte in the solution

**Example of Molecular spectroscopy,**

- Ultraviolet and Visible Spectrophotometries (UV/VIS)
- Infrared Spectrophotometries (IR)
- Fluorescence/Phosphorescence Spectrophotometries (Flu/Phs)

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## Electromagnetic radiation (em)

*The Electromagnetic Spectrum*

| | Gramma rays | X-rays | Ultraviolet | Visible light | Infrared | Micro waves | Radio waves |
|---|---|---|---|---|---|---|---|
| Wavelength λ (nm) | < 0.001 | 1-10 | 190–400 | 400-750 | 750-0.1 | 0.1-100 | 100-1000 |
| | Short wavelength → Long wavelength | | | | | | |

In Ultraviolet-visible spectrometry, the analysis based on the use of electromagnetic radiation in the wavelength region of **190 to 800 nm**

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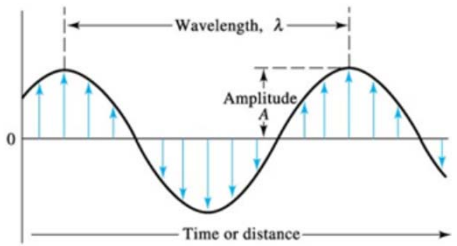
## Electromagnetic radiation (em)

**Wave properties**

It is characterized with ***wavelength*** and ***frequency***.

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## Electromagnetic radiation (em)

**General terms**

| | |
|---|---|
| A = | **Amplitude**<br>**the length of the electrical vector at the max. in the wave** |
| P = | **Period**<br>**the time required for the passage of successive max. (or min.) through a fixed point in space** |
| f = | **Frequency (unit: Hz or $s^{-1}$)**<br>**no. of oscillations of the field per second** |
| λ = | **Wavelength**<br>**linear distance between successive max. and min. of a wave** |
| | $v = f\lambda$<br>**where v = velocity of light in vacuum ( $3 \times 10^8$ m/s)** |
| ν = | **Wavenumber**<br>**the number of wavelengths per unit distance, $cm^{-1}$**<br>**Wavenumber = 1 / λ** |

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## 4.2 Principle of molecular absorption

**4.2.1 Molecular absorption spectrum**

**4.2.2 Type of energy transitions**

**4.2.3 Beer-Lambert Law**

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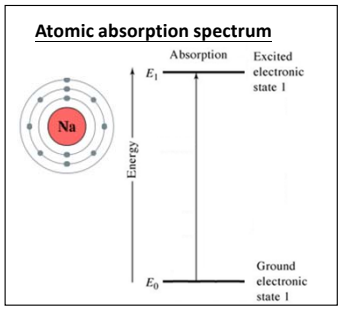
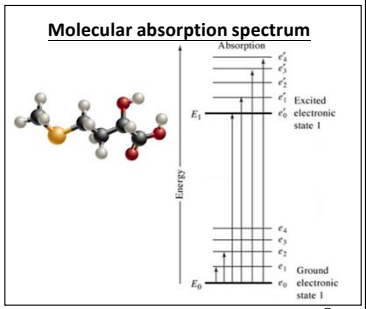
## 4.2.1 Molecular absorption spectrum

- **more complex** than atomic absorption spectrum

→ there are **more energy states** in molecules

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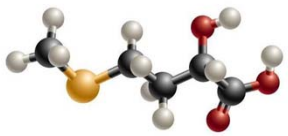
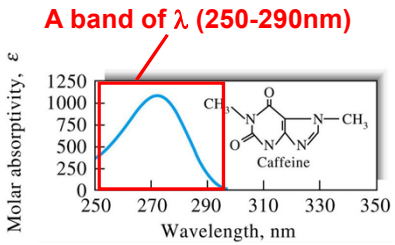
## 4.2.1 Molecular absorption spectrum

- **band absorption spectrum**
- e.g. from 250 to 290 nm
- ∵ molecules absorb in a **continuous range of wavelengths**

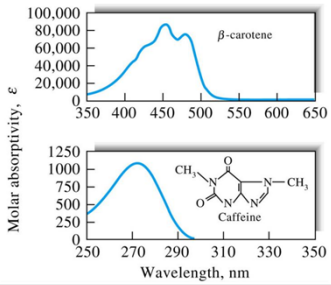





## 4.2.1 Molecular absorption spectrum

Molecular absorption of UV-Vis involves

- **bonding electrons**,
- the **absorption band** can be used for
  - identification of different **functional groups**

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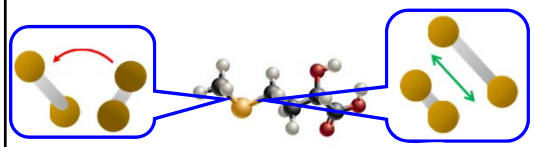
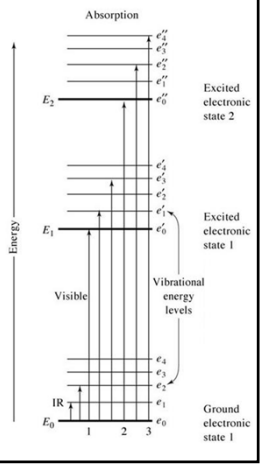
## 4.2.2 Types of energy transition

- unexcited molecule **absorb specific wavelength, $\lambda_{specific}$**
  - ground state **bonding electron** transits to a higher **excited**
    **(i) electronic,**
    **(ii) vibrational or**
    **(iii) rotational states**



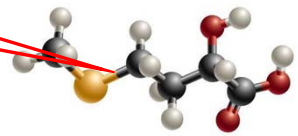


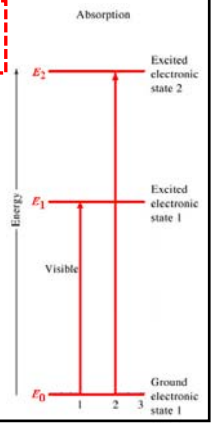

## 4.2.2 Types of energy transition

Energy (E) being absorbed by the unexcited molecule:

★ **$E = E_{electronic} + E_{vibrational} + E_{rotational}$**

**$E_{electronic}$**

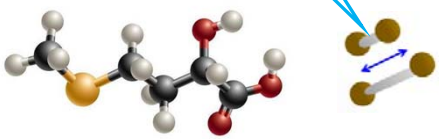


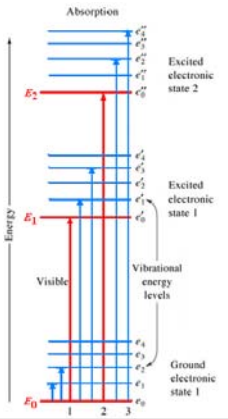


## 2. Principle of molecular absorption

Energy (E) being absorbed by the unexcited molecule:

$E = E_{electronic} + E_{vibrational} + E_{rotational}$



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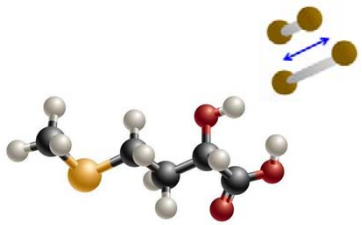
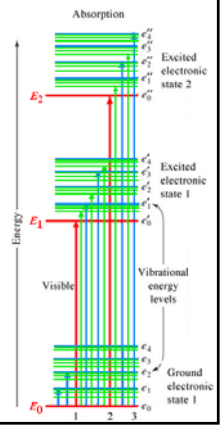
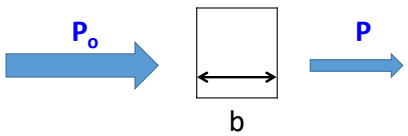





## 4.2.3 The Beer-Lambert Law

**Beer's law** states that

- the **intensity** of a beam of ray of **monochromatic light (single wavelength)** **decreases exponentially** as the **concentration** of the absorbing medium **increases**.




$P_o$ - intensity of light ***entering*** solution
P - intensity of light ***leaving*** solution
*b - length of absorbing layer (pathlength)*

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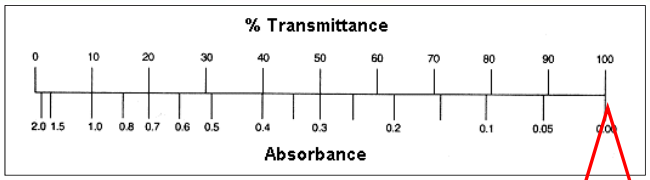
## 4.2.3 The Beer-Lambert Law




**Transmittance, $T = P/P_o = 10^{-k'b}$**
**Absorbance, $A = -\log T$**
**Absorbance, $A = \log (P_o/P) = k'b \rightarrow A \propto b$**

**100% transmittance = zero absorbance**

**P = intensity of light leaving solution**
**$P_o$ = intensity of light entering solution**
**b = length of absorbing layer (pathlength)**
**k' = constant for a particular solution**

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## 4.2.3 The Beer-Lambert Law

- the absorbance of the analyte is proportional to

  (i) the concentration of the analtyte

  (ii) the path length of the sample cell

**Absorbance, A = $\varepsilon$ bc**

| | |
|---|---|
| $\varepsilon$ | the molar absorptivity of the solution in ***$cm^{-1}mol^{-1}L$*** |
| b | path length of the sample cell in ***cm*** |
| c | concentration of the solution in ***mol/L or M*** |

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## 4.2.3 The Beer-Lambert Law

$$\text{Absorbance, } A = \varepsilon bc = \log_{10}\left(\frac{P_o}{P}\right)$$

| | |
|---|---|
| $\varepsilon$ | the molar absorptivity of the solution in ***$cm^{-1}mol^{-1}L$*** |
| b | path length of the sample cell in ***cm*** |
| c | concentration of the solution in ***mol/L or M*** |
| $P_o$ | intensity of light entering solution |
| P | intensity of light leaving solution |

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## 4.2.3 The Beer-Lambert Law

**Transmittance VS Absorbance**

$$\text{Absorbance, } A = \log_{10}\left(\frac{P_o}{P}\right) \qquad \text{Transmittance, } T = \frac{P}{P_o}$$

$$A = -\log_{10} T$$

**100% transmittance = zero absorbance**

★ **Calculation of**
- **absorbance**
- **molar absorptivity**
- **path length**
- **transmittance(%)**

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## 4.2.3 The Beer-Lambert Law

The molar absorptivity ($\varepsilon$)

- is a measurement of how strongly a chemical species absorbs light **at a given wavelength**.

It is an **intrinsic property** of the species.

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## 4.2.3 The Beer-Lambert Law

**Example**

A solution with concentration 7.25 $\times 10^{-5}$ M has a transmittance of 44.1% when measured in a 2.10 cm cell at wavelength of 525 nm. Calculate:
(a) the absorbance of this solution
(b) the molar absorptivity of this solution.

**Solutions**
(a) A = -log T = -log 0.441 = -(-0.3554) = 0.355
(b) Molar absorptivity
= A/bc
= 0.3554 / (2.1 cm $\times$ 7.25 $\times 10^{-5}$ mol $L^{-1}$)
= 2.33 $\times$ $10^{3}$ $Lmol^{-1}$ $cm^{-1}$

## 4.2.3 The Beer-Lambert Law

**Classwork**

A solution placed in a 1.0 cm sample cell and 70% of light is transmitted.
If the molar absorptivity of the solution is 2.0 $cm^{-1}g^{-1}L$.
What is the concentration of the solution?

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## 4.2.3 The Beer-Lambert Law

**Classwork (Answer)**

b = _____ cm
transmitted, T = _____ % = 0.7
molar absorptivity, ε = _____$cm^{-1}g^{-1}L$.

A = -log T = ε bc

-log _____ = (_____) (_____ ) (c)

_____ = (_____) (c)

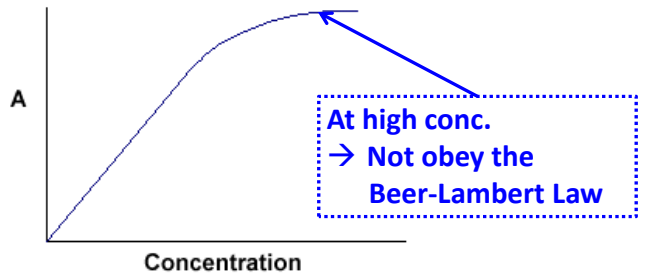
c = _____ / _____

c = _____ g/L

## 4.2.3 The Beer-Lambert Law

**Limitation of Beer-Lambert Law**

Absorbance, A = εbc

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## 4.2.3 The Beer-Lambert Law

**Limitation of Beer-Lambert Law**

**Reference ONLY!!**

At **concentration > 0.01 M**, the average distances between ions or molecules of the absorbing species are diminished to the point where each particle affects the charge distribution and thus the extent of absorption.

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## 4.3 Instrumentation of UV/VIS Spectrophotometer

**4.3.1 main components & block diagram**

**4.3.2 radiation sources**

**4.3.3 wavelength selector**

**4.3.4 sample**

**4.3.5 detector**

**4.3.6 readout device**

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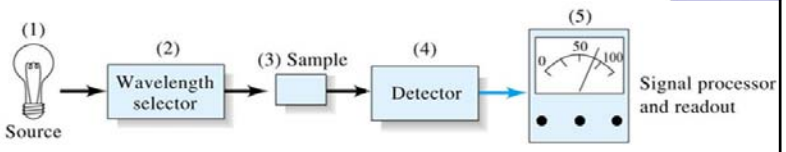
## 4.3.1 Main components & block diagram




**5 essential components of spectrometric instruments**

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## 4.3.1 Main components & block diagram

★ *block diagram* of UV/VIS spectrophotometers

**Radiation Source** → **Wavelength selector** → **Sample** → **Detector** → **Readout device**

**5 essential components of spectrometric instruments**

*What is a Block diagram?*

**Reference ONLY!!**

- *usually constructed to illustrate the major components of an analytical instrument.*
- *In a block diagram, there is no need to draw the actual structure of the instrument component*

## 4.3.1 Main components & block diagram

5 main components in UV/VIS spectrophotometer

1. Radiation sources – stable light source
2. A wavelength selector - select interested wavelength
3. Sample cell - One or two sample cell
4. A radiation detector - converts radiant energy to measurable electrical signal.
5. A signal processing and readout device.

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## 4.3.2 Radiation Source

Radiation Source → Wavelength selector → Sample → Detector → Readout device

**Properties:**

- **Sufficient power**
- **Stable**

**Ideal light source →**
**• low noise**
**•Long-term stability**

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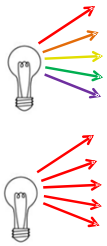
## 4.3.2 Radiation Source

**Two kinds of radiation sources:**

| Continuum source | Line source |
|---|---|
| • Produce broad wavelength | • Emit a few discrete lines |
| • **NOT specific wavelength** | • **Relatively specific** |



Wavelength of different radiation sources

https://goo.gl/images/nAqWAW

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## 4.3.2 Radiation Source

**Two kinds of radiation sources:** **Reference ONLY!!**

| Continuum source | | Line source |
|---|---|---|
| In UV region | Visible & near IR region | In UV/VIS regions |
| For example:<br>• Deuterium<br>• Hydrogen lamp<br>• Gas filled arc lamps,<br>• Light emitting diodes | For example:<br>• Tungsten filament lamp | Example:<br>• Mercury and sodium vapor lamps |

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## 4.3.3 Wavelength selector


- A device to **isolates a desired** wavelength for measurement
- **No selector** is capable of producing radiation of a **single wavelength.**
- Instead, the output of such a device is a range of **contiguous wavelengths** called a band.

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## 4.3.3 Wavelength selector

https://goo.gl/images/L2TUKi

https://goo.gl/images/f91qHg

Interference filter

https://goo.gl/images/AqgCqv

Monochromator

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## 4.3.3 Wavelength selector

1. **Filters**

a) **Interference filter**

b) **Absorption filter**

**2. Monochromator → most common**

**a) Prism**

**b) Reflection grating**

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## 4.3.3 Wavelength selector

1. **Filters**

- **Provide low resolution wavelength selection (i.e. bandwidth is larger)**
- ***Wavelength cannot* be *change* continuously**
- **For *quantitative* analysis**

**Example**

a) **Interference filter:** for UV, visible & IR range

b) **Absorption filter:** for visible range

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## 4.3.3 Wavelength selector

1. **Filters**

a) **Interference filter**

https://goo.gl/images/rafDj4

b) **Absorption filter**

https://goo.gl/images/Uy3UDS

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## 4.3.3 Wavelength selector

2. **Monochromator**

- **Provide higher resolution wavelength selection (i.e. bandwidth is narrow)**
- ***Wavelength can be change*** **continuously**
- **For *qualitative and quantitative* analysis**

**Example**

a) **Prism**

b) **Reflection grating**

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## 4.3.3 Wavelength selector

2. **Monochromator:**

Two basic designs:

**(a) Prism**

https://goo.gl/images/ayiiyL

**(b) Reflection grating**

https://goo.gl/images/aBeSfq

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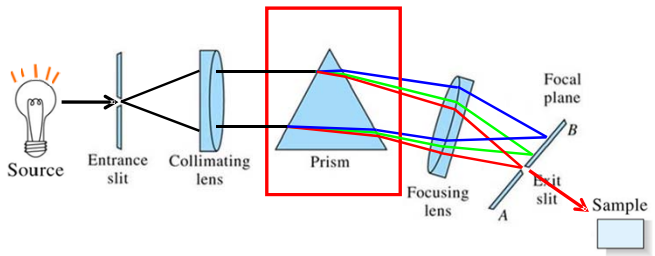
## 4.3.3 Wavelength selector

2. **Monochromator**

a) **Prism**

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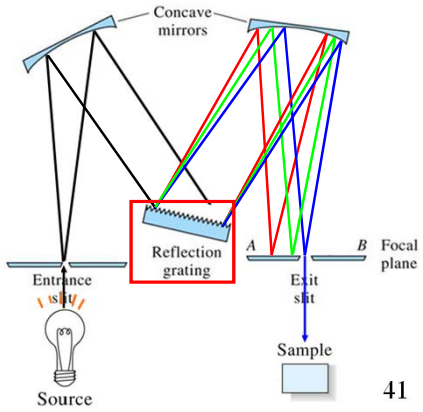
## 4.3.3 Wavelength selector

2. **Monochromator**

b) **Reflection grating**

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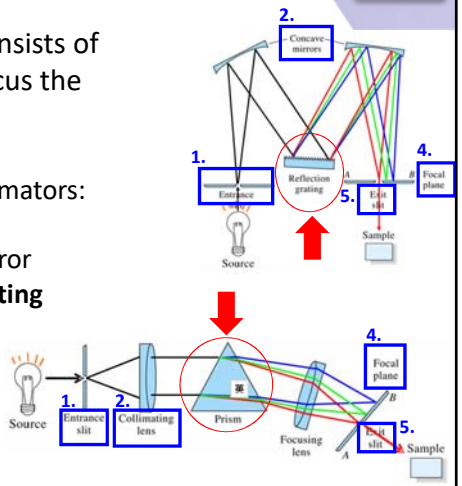
## 4.3.3 Wavelength selector

2. **Monochromator:**

- A monochromator consists of *lenses or mirror* to focus the radiation,

Components of Monochromators:

1. Entrance slit
2. Collimating lens or mirror
3. **prisms / reflection grating**
4. Focal plane
5. Exit slit

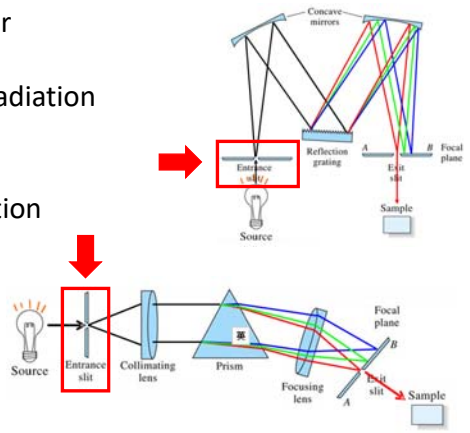



## 4.3.3 Wavelength selector

1. **Entrance slit:**

**Reference ONLY!!**

- protect from dust or corrosive fumes
- restrict unwanted radiation
- control the spectral bandwidth of radiation emitted from the monochromator,

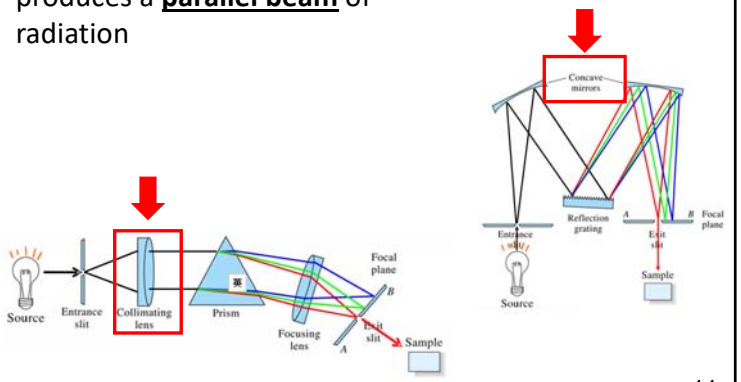



## 4.3.3 Wavelength selector

2. **Collimating lens or mirror**

**Reference ONLY!!**

- produces a **parallel beam** of radiation

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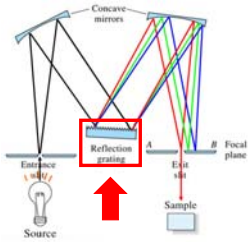
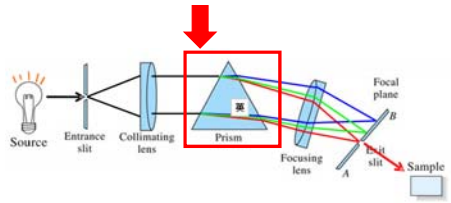
## 4.3.3 Wavelength selector

**3. Prism or reflection grating**

- disperses the radiation to different wavelengths
- by rotating the prism / reflection grating

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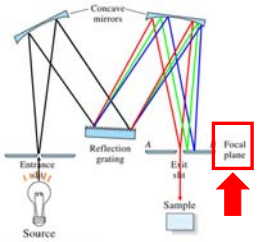
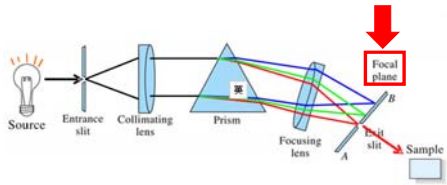
## 4.3.3 Wavelength selector

**4. Focal plane**

Reference ONLY!!

- reforms the image and focuses it on a planar surface on the exit slit

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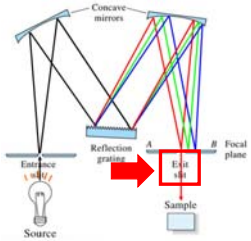
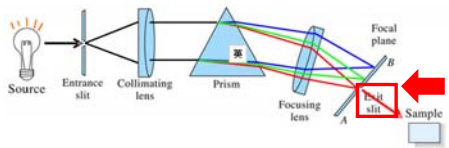
## 4.3.3 Wavelength selector

**5. Exit slit**

Reference ONLY!!

- protect from dust or corrosive fumes
- restrict unwanted radiation
- control the spectral bandwidth of radiation emitted from the monochromator

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## 4.3.3 Wavelength selector

**Monochromator**

**a) Prism**

- Prism works satisfactory in the UV/VIS region.
- By rotation of prism,
  - different wavelengths can pass the slit.

Diagram of the prism

https://goo.gl/images/Z3he2D

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## 4.3.3 Wavelength selector

**Monochromator**

**b) Reflection grating**

- cheaper
- provides **better wavelength separation**
- grating consists of closely spaced grooves
- reflection on the surface due to a thin layer of **Al, Au or Pt** is coated

UV-Vis from 300 to 2000 grooves/mm
infrared from 10 to 200 grooves/mm

Diagram of the reflecting grating

https://goo.gl/images/k4n8bB

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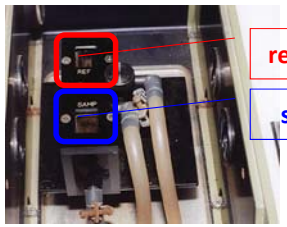
## 4.3.4 Sample

Radiation Source → Wavelength selector → Sample → Detector → Readout device

**Sample Cell**

**Sample compartment**

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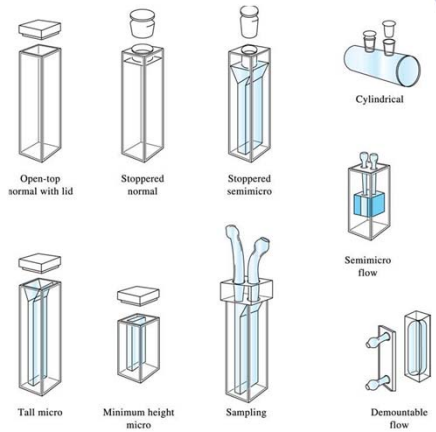
## 4.3.4 Sample

**Different Sample holder**

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## 4.3.4 Sample

**Sample holder:** Cells or cuvettes

- Materials should be transparent in the spectral region of interest
- **UV region:**
  - **Quartz or fused silica**
- **visible region:**
  - **Plastic cell**


https://goo.gl/images/4Ay9GU

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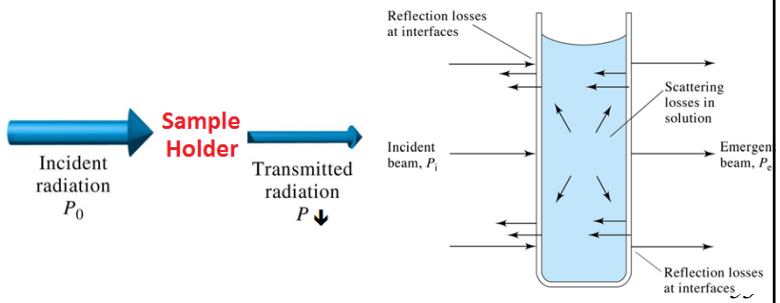
## 4.3.4 Sample

**Blank measurement:**

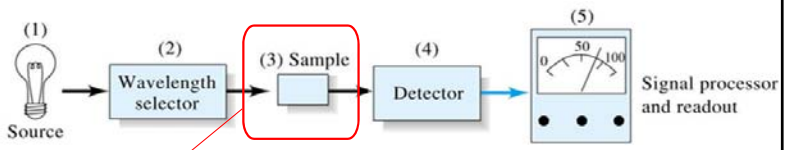
- **To compensate for the effects of absorption by the container, reflection, and also any scattering effects**




## 4.3.4 Sample

**Blank measurement:**

- To compensate the effect power beam of **analyte solution** is compared with solvent only




**Measure:**
1. Blank (solvent)
2. Standard (standard + solvent)
3. Sample (sample + solvent)

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## 4.3.5 Detector

Radiation Source → Wavelength selector → Sample → Detector → Readout device

- **Detection of photons**

★ ***Different Types of photon detectors:***

**3.5.1 Single channel**
- (i) photo tube
- (ii) photomultiplier tube (PMT)

**3.5.2 Multichannel**
- (i) diode array detector(DAD)

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## 4.3.5 Detector

Radiation Source → Wavelength selector → Sample → Detector → Readout device

- **Convert radiant energy into electrical signal**

**An ideal detector**
- **High sensitivity**
- **High signal to noise ratio**
- **Stabile and fast response**
- **Zero output signal in the absence of illumination**

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## 4.3.5 Detector

1. **Single channel:**
- **measure single wavelength at a time**

**Example:**
**(i) Phototube**
**(ii) photomultiplier tube (PMT)**

2. **Multichannel**
- **Measure different the wavelengths simultaneously**

**Example:**
**(i) diode array detector (DAD)**

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## 4.3.5 Detector

1. **Single channel:**

**(i) Photo tube**

- **An active surface (cathode)**
- **radiation (photon beam)**
- **causes emission of electrons**

Diagram of the photo tube

https://goo.gl/images/v2vVtQ

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## 4.3.5 Detector

1. **Single channel:**

**(ii) photomultiplier tube (PMT)**

- **Advantages over the ordinary phototube**

Photomultiplier tube animation
https://www.youtube.com/watch?v=f61eMq4Wg4w

Diagram of the photomultiplier tube (PMT)

https://goo.gl/images/Nqo3nD

## 4.3.5 Detector

**Principle of photomultiplier (PMT)**

- **Photo cathode surface is similar to the surfaces of the phototubes**
- **Emit electrons when exposed to radiation**
- **Contains additional electrode called dynodes**

Photomultiplier tube animation
https://www.youtube.com/watch?v=f61eMq4Wg4w

Diagram of the photomultiplier tube (PMT)

https://goo.gl/images/Nqo3nD

## 4.3.5 Detector

**Principle of photomultiplier (PMT)**

- **Dynodes 1** is maintained at a **positive potential** higher than cathode
- Upon striking the dynodes, **each photoelectron** causes emission of **several additional electrons**
- then, **electrons** are accelerated toward **dynode 2**,
  - which is also at higher positive potential than dynode 1

Photomultiplier tube animation
https://www.youtube.com/watch?v=f61eMq4Wg4w

Diagram of the photomultiplier tube (PMT)

https://goo.gl/images/Nqo3nD

## 4.3.5 Detector

**Principle of photomultiplier (PMT)**

- this process has been **repeated** through dynode 1 to dynode 9
- $10^6$ to $10^7$ electrons have been formed for each incident photon
- Cascade is finally collected at the anode
- The resulting current is **amplified** and **measured**

Photomultiplier tube animation
https://www.youtube.com/watch?v=f61eMq4Wg4w

Diagram of the photomultiplier tube (PMT)

https://goo.gl/images/Nqo3nD

## 4.3.5 Detector

**2. Multichannel**

- **Measure different wavelengths simultaneously**
- **Full spectrum can be generated**
- **Can determine more than one chemical compound simultaneously**

**Example:**

**(i) diode array detector (DAD)**

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## 4.3.6 Readout device

**Radiation Source** → **Wavelength selector** → **Sample** → **Detector** → **Readout device**

- electronic device that amplifies the electrical signal from the detector
- Perform mathematical operation

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## 4.4 Different design of UV-VIS Spectrophotometer

**4.4.1 Single-beam design**

**4.4.2 Double beam design**

- **In space (using *beam splitter*)**
- **In time (using *sector mirror*)**

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## 4.4 Different design of UV-VIS Spectrophotometer

**4.4.1 Single-beam design**

- with **alternate** measurement of
  - blank
  - standards
  - samples
- usually not for spectra scanning.
- Cost – cheaper than double beam design

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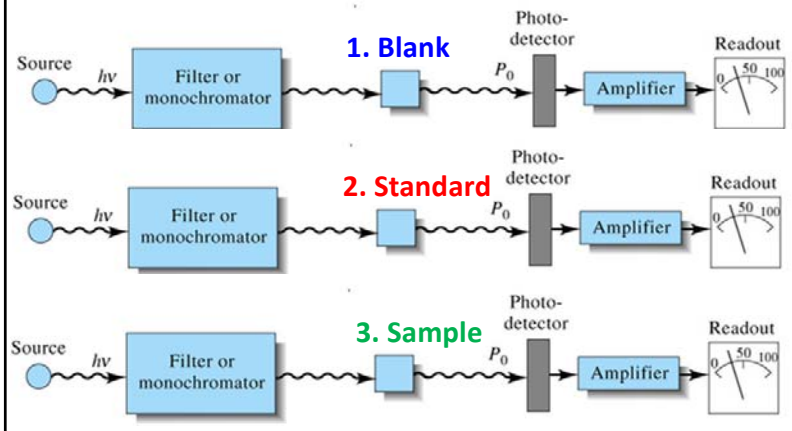
## 4.4 Different design of UV-VIS Spectrophotometer

**4.4.1 Single-beam design**

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## 4.4 Different design of UV-VIS Spectrophotometer

**4.4.1 Single-beam design**

**Blank measurement:**

- To compensate the effect power beam of **analyte solution** is compared with solvent only

**Radiation Source** → **Wavelength selector** → **Sample** → **Detector** → **Readout device**

**Measure:**
**1. Blank (solvent)**
**2. Standard (standard + solvent)**
**3. Sample (sample + solvent)**

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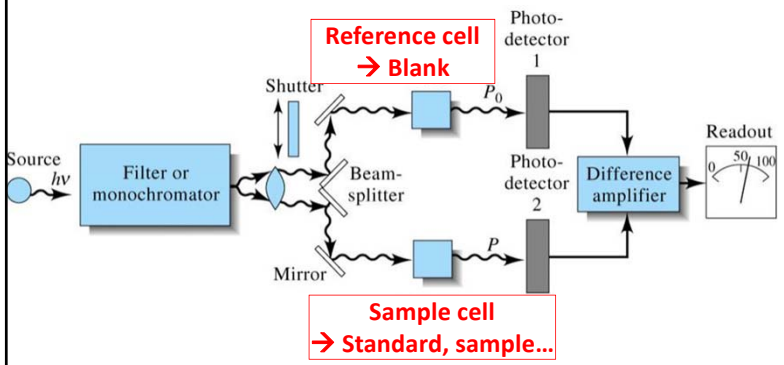
## 4.4 Different design of UV-VIS Spectrophotometer

### 4.4.2 Double beam design

- measurement of sample and blank simultaneously

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## 4.4 Different design of UV-VIS Spectrophotometer

### 4.4.2 Double beam design

★ ***Advantages of double beam design:***

- compensate the fluctuations in the radiation source
- reduce the fluctuations / drift of the detector
- reduce the effect of wavelength instability of the spectrophotometer

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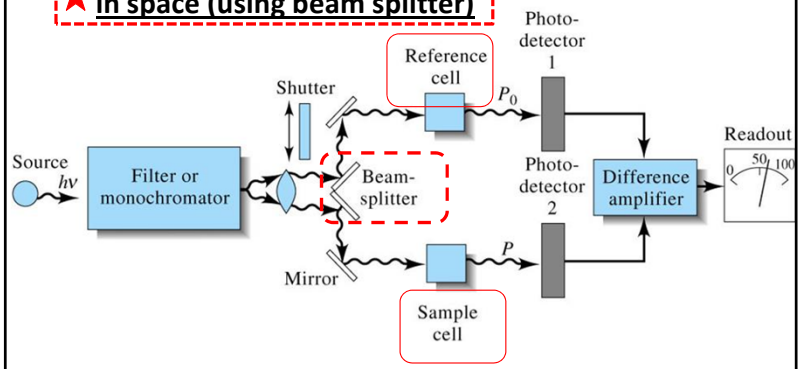
## 4.4 Different design of UV-VIS Spectrophotometer

### 4.4.2 Double beam design

★ in space (using beam splitter)

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## 4.4 Different design of UV-VIS Spectrophotometer

### 4.4.2 Double beam design

★ in space (using beam splitter)

- two beams are formed by a V-shaped mirror called a beam splitter.
- One beam passes through the reference solution to a photodetector and
- the second beam passess simultaneously through the sample to a second matched photodetector.

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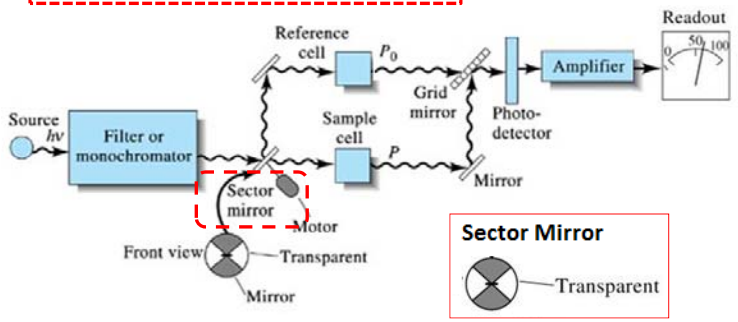
## 4.4 Different design of UV-VIS Spectrophotometer

### 4.4.2 Double beam design

★ **in time (using sector mirror)**

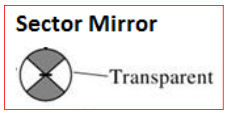





## 4.4 Different design of UV-VIS Spectrophotometer

### 4.4.2 Double beam design

★ **in time (using sector mirror)**




- the **beams** are **separated in time**
- by **rotating sector mirror** that
- directs the entire beam through the reference cell
- and then through the sample cell.



## 4.5 Analysis using UV-VIS Spectrophotometers

**4.5.1 Qualitative analysis using UV/Vis**

**4.5.2 Quantitation analysis using UV/Vis**

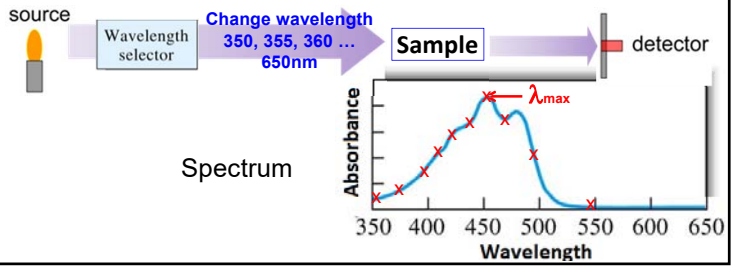
**4.5.3 Application of UV/Vis**

**4.5.4 More about quantitative analysis**



## 4.5.1 Qualitative analysis using UV/VIS

- changing the wavelength
- e.g. from 350 – 650nm
  - measure the absorbance
- **UV spectrum (x-axis – wavelength, y-axis absorbance)**
- Compare the UV/Vis spectrum of sample/ standard/wavelength maximum



## 4.5.1 Qualitative analysis using UV/VIS

Limitation in qualitative analysis using UV-Vis

- number of absorption maxima and minima are relatively few
- difficult to identification the unknown compound

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## 4.5.2 Quantitative analysis using UV/VIS

★ 1. Select the **wavelength maximum λmax**
i.e. the wavelength with the maximum absorbance

2. **Fixed** the wavelength at **λmax**
3. **Measure the absorbance** of blank, calibration standards & sample
4. determine the concentration of sample


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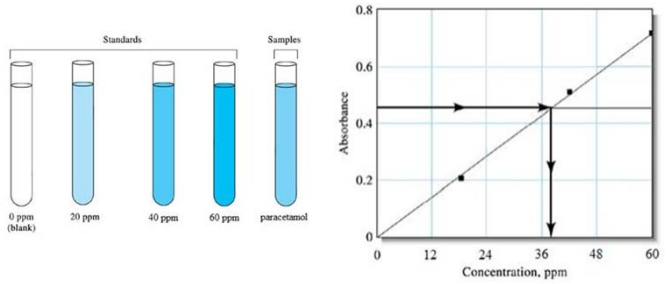
## 4.5.2 Quantitative analysis using UV/VIS

5. **Plot calibration curve**
   - **X-axis concentration & Y-axis absorbance**
6. Determination the concentration of the sample

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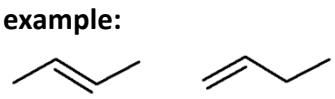
## 4.5.3 Application of UV/VIS

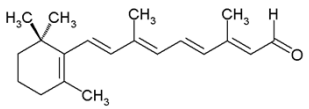
★ **Ultra-violet region**

- Mainly for analytes with ***conjugated double bonds*** or with ***aromatic rings***
- molecules containing **unsaturated functional group**
- ***Chromophore***

**For example:**





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## 4.5.3 Application of UV/VIS

**Visible region**

- Mainly for analytes with ***colored***
- Or ***derivatisation with coloring agent***

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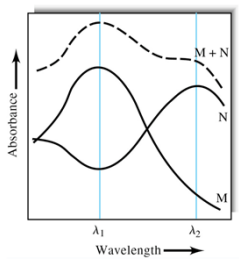
## 4.5.4 More about quantitative analysis

- **Analysis of mixtures of absorbing substances**
- Total absorbance of a solution at any given wavelength is equal to the sum of the absorbance of the individual components in the solution

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## 4.5.4 More about quantitative analysis

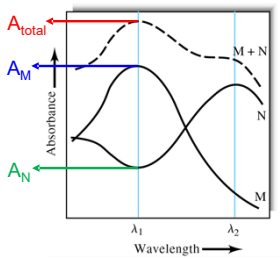
- **Analysis of mixtures of absorbing substances**

At $\lambda_1$:

$A_{total} = A_M + A_N$

$A_M = \varepsilon_{M_{\lambda_1}} bc_M$

$A_N = \varepsilon_{N_{\lambda_1}} bc_N$

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## 4.5.4 More about quantitative analysis

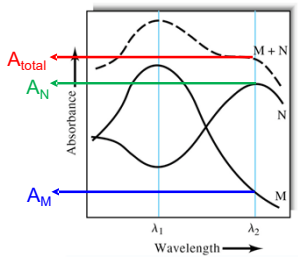
- **Analysis of mixtures of absorbing substances**

At $\lambda_2$:

$A_{total} = A_M + A_N$

$A_M = \varepsilon_{M_{\lambda_2}} bc_M$

$A_N = \varepsilon_{N_{\lambda_2}} bc_N$

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## 4.5.4 More about quantitative analysis

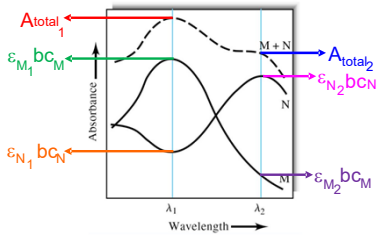
- **Analysis of mixtures of absorbing substances**

At $\lambda_1$:

$A_{total_1} = \varepsilon_{M_1} bc_M + \varepsilon_{N_1} bc_N$

At $\lambda_2$:

$A_{total_2} = \varepsilon_{M_2} bc_M + \varepsilon_{N_2} bc_N$




By measuring $A_1$ and $A_2$ and known $\varepsilon_{M_1}$, b, $\varepsilon_{N_1}$, $\varepsilon_{M_2}$, $\varepsilon_{N_2}$
$c_N$ and $c_M$ can be found



## 4.5.4 More about quantitative analysis

★ **Example**:

The absorbance of solution X and solution Y is determined using UV/Vis spectrophotometer.

The results is shown in below:

| Solution | Concentration (M) | Sample Cell length (cm) | Absorbance | |
|---|---|---|---|---|
| | | | 400 nm | 500 nm |
| X | $2 \times 10^{-4}$ M | 1.0 cm | 0.800 | 0.760 |
| Y | $4.25 \times 10^{-5}$ M | 1.0 cm | 0.34 | 0.085 |

a) Calculate the molar absorptivity (ε) of solution X and solution Y at 400 nm and 500 nm respectively.

## 4.5.4 More about quantitative analysis

★ **Example (Answer)**:

Using A = ε b c ➔ Molar absorptivity, ε = A / ( b × c )

For solution X (at 400nm), molar absorptivity (ε)
= 0.8 / (1.0 cm × $2 \times 10^{-4}$ M) = 4000 $M^{-1}cm^{-1}$
For solution X (at 500nm), molar absorptivity (ε)
= 0.760 / (1.0 cm × $2 \times 10^{-4}$ M) = 3800 $M^{-1}cm^{-1}$
For solution Y (at 400nm), molar absorptivity (ε)
= 0.34 / (1.0 cm × $4.25 \times 10^{-5}$ M) = 8000 $M^{-1}cm^{-1}$
For solution Y (at 500nm), molar absorptivity (ε)
= 0.085 / (1.0 cm × $4.25 \times 10^{-5}$ M) = 2000 $M^{-1}cm^{-1}$

## 4.5.4 More about quantitative analysis

★ **Example**:

Solution X & Y then mixture together and analyze using UV/VIS spectrophotometer.

The results is shown in below:

| Mixture of solution X & solution Y | Sample Cell length (cm) | Absorbance | |
|---|---|---|---|
| | | 400 nm | 500 nm |
| | 2.5 cm | 0.680 | 0.520 |

b) Calculate the concentration (in M) of solution X and solution Y in the mixture.

## 4.5.4 More about quantitative analysis

★ **Example (Answer)**:

| | Absorbance | Sample Cell length (cm) | **molar absorptivity (ε)** | |
|---|---|---|---|---|
| | | | Solution X | Solution Y |
| 400nm | 0.680 | 2.5 cm | 4000 | 8000 |
| 500nm | 0.520 | 2.5 cm | 3800 | 2000 |

$\mathbf{C_x}$ & $\mathbf{C_x}$: concentration of solution X & Y in the mixture

At 400nm, $4000(2.5)\ \mathbf{C_x} + 8000\ (2.5)\ \mathbf{C_y} = 0.680$

$4000\ \mathbf{C_x} + 8000\ \mathbf{C_y} = 0.680 / 2.5$

$4000\ \mathbf{C_x} + 8000\ \mathbf{C_y} = 0.272$

At 500nm, $3800(2.5)\ \mathbf{C_x} + 2000\ (2.5)\ \mathbf{C_y} = 0.520$

$3800\ \mathbf{C_x} + 2000\ \mathbf{C_y} = 0.208$

## Notes



## 4.5.4 More about quantitative analysis

Solving the two equation:

$4000\ \mathbf{C_x} + 8000\ \mathbf{C_y} = 0.272$ --------- equation (1)

$3800\ \mathbf{C_x} + 2000\ \mathbf{C_y} = 0.208$ --------- equation (2)

Equation (2) × 4

$15200\ \mathbf{C_x} + 8000\ \mathbf{C_y} = 0.832$ --------- equation (3)

equation (3) - equation (1)

$11200\ \mathbf{C_x} = 0.56$

$\mathbf{C_x} = 5 \times 10^{-5}$ M

∴concentration of solution X in the mixture = $5 \times 10^{-5}$ M and concentration of solution Y in the mixture = $9 \times 10^{-6}$ M

put $\mathbf{C_x} = 5 \times 10^{-5}$ into the equation

$\mathbf{C_y} = 9 \times 10^{-6}$ M

## Notes